Data Evaluation Record on the Acute Toxicity of Hoe 099730 Technical to Algae, *Scenedesmus subspicatus*

EPA MRID Number 48444813

Data Requirement:

EPA DP Barcode	345709
EPA MRID	48444813
EPA Guideline	850.5400

Test material: **Hoe 099730 Technical**

Purity: 92.4%; applied as an aqueous solution with a concentration of 35.7%

Common name
Chemical name: IUPAC disodium L-2-acetamido-4-methylphosphinato-butyrate
CAS name
CAS No.
Synonyms N-acetyl-glufosinate (NAG)

Primary Reviewer: Moncie Wright
Staff Scientist, Cambridge Environmental Inc.
Signature: Moncie V Wright
Date: 7/26/11

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.
Signature: Teri S Myers
Date: 10/19/11

Primary Reviewer: Catherine Aubee
Biologist, US EPA/OPP/EFED/ERBIV
Signature: C Aubee
Date: 1 June 2012

EPA PC Code 128850

Date Evaluation Completed: 01-06-2012

CITATION: Heusel, R. 1996. Hoe 099730 - substance, technical – Code: Hoe 099730 00 ZC92 0001): Effect to *Scenedesmus subspicatus* (green algae) in a growth inhibition test (method OECD). Unpublished study performed and sponsored by Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany. Study completed March 13, 1996.

Data Evaluation Record on the Acute Toxicity of Hoe 099730 Technical to Algae, *Scenedesmus subspicatus*

EPA MRID Number 48444813

EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to **Hoe 099730 Technical** (or NAG), a transformation product of the herbicide glufosinate-ammonium, under static conditions at nominal concentrations of 0 (negative control), 100, 180, 320, 560, and 1000 mg aqueous solution/L, which are equivalent to 0 (negative control), 35.7, 64.3, 114, 200, and 357 mg ai/L. Measured concentrations were only obtained for a low (35.7 mg ai/L), middle (114 mg ai/L), and high concentration (357 mg ai/L) and recoveries ranged from 86 to 118% of nominal. As a result, toxicity values in this study calculated by the reviewer were based on a combination of the corrected nominal concentrations and the available measured concentrations.

The most sensitive endpoint could not be determined due to a lack of toxicity in this study, resulting in overall NOAEC and EC_{50} values of 384 and >384 mg ai/L, respectively (based on measured concentration). The % growth inhibition of cell density in the treated algal culture as compared to the control ranged from -21 to 13%.

No phytotoxicity was reported.

This toxicity study is classified as scientifically sound and is classified as **supplemental**. Although consistent with OECD test guideline 201, it does not satisfy OCSPP guideline 850.5400 because the algae in controls had not reached the logarithmic growth phase by test termination and the light intensity was much higher than what is recommended in the OCSPP guideline. These deviations may affect the nature of the growth curve and the concentration-response, thereby limiting the utility of the information in EPA risk assessment. However, EFED is not requesting additional algal toxicity tests with glufosinate degradates at this time.

Results Synopsi

Test Organism: *Scenedesmus subspicatus*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test procedure followed the guidelines of the Organization for Economic Cooperation and Development (OECD), Guideline No. 201: Alga, Growth Inhibition Test (1984). The study methods and results were evaluated according to U.S. EPA OPPTS 850.5400: Algal Toxicity, Tiers I and II and OECD No. 201, and differences and/or similarities were described. Two deficiencies and deviations from OPPTS 850.5400 and OECD 201 were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

2. Analytical verification was not performed for all test concentration levels; OPPTS guidelines suggest that the concentration of the test material in the test vessels should be determined at the beginning and end of the test. OECD guidelines also recommend verifying the test concentrations at the beginning of the test and also verifying that those concentrations have been maintained during the test.

3. The test temperature ranged from 24.6 to 25.9°C; while the species tested is not included in either OPPTS or OECD guidelines, this temperature is still higher than recommended for similar algal species (OPPTS: 24°C and OECD: 21-24°C).

4. The physico-chemical properties of the test material were not reported; OECD guidelines suggest that this information be reported. OPPTS guidelines do not address this topic.

5. The pH of the control ranged from 7.9 to 9.9 and in the test solutions ranged from 7.6 to 9.9; OPPTS guidelines suggest a pH of 7.5 ± 0.2 for similar algal species. Additionally, OECD guidelines suggest that the control pH not vary by more than 1.5 units.

The deficiencies and deviations do not substantively impact the scientific soundness of the study, but they may limit its utility in risk assessment.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice as set forth in Appendix II to OECD Council Decision C(81)30 of 12 May 1981, endorsed for use in the European Union by Directive 87/18 of 18 December 1986 and implemented at the national level as: Good Laboratory Practice, German Chemicals Act, §19a, Bundesgesetzblatt 1703-1732, 25 July 1994.

A. MATERIALS:

1. Test material **Hoe 099730 technical**

Description: Clear liquid

Lot No./Batch No. : Not reported

Purity: 92.4% w/w; supplied as an aqueous solution with a concentration of 35.7%

Stability of compound under test conditions: Analytical verification was only performed for the low, middle, and high test level solutions. At time 0, recoveries ranged from 86 to 114% of nominal concentrations. At 72 hours (test termination), recoveries ranged from 95 to 118% of nominal. The test material appeared to be stable under the test conditions.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of Hoe 099730.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Green algae; *Scenedesmus subspicatus* CHODAT

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 86.81 SAG
Source: In-house cultures originally obtained from the Collection of Algal Cultures, Institute of Plant Physiology, University of Gottingen, Gottingen, Germany
Age of inoculum: 3 days
Method of cultivation: Algae were cultivated in nutrient medium

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test (dilution water, temperature, agitation, photoperiod, and light intensity) Not reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
<u>Test system</u> Static/static renewal Renewal rate for static renewal	 Static N/A	 *EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	The test vessels were placed in a waterbath positioned on an electric shaker.	
Duration of the test	72 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
<u>Test vessel</u> Material: *(glass/stainless steel)*	Glass	

Parameter	Details	Remarks
		Criteria
Size: Fill volume:	300 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 7.8-7.9 8.5-9.9 Yes $NaHCO_3$ N/A	Control pH: 7.9-9.9 *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water 8.0 N/A Filtered by ultrafiltration, ion exchange, and a charcoal unit Not reported Not reported Not reported Not reported Not reported	The deionized water was used to create reagent grade water that was used to prepare the algal medium. *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test material (0.1 mg) was dissolved in nutrient medium to create a primary stock solution. The solution was shaken well and defined amounts were pipetted proportionally into graduated cylinders. The flasks were then filled up to 90 mL with nutrient medium. Pre-culture (0.53 mL) was added to the vessels, which were then filled up to 100 mL with nutrient medium.	
Aeration or agitation	Agitation; 100 rpm	
Initial cells density	1 x 10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal (unadjusted for purity): Nominal (adjusted for purity): Measured:	0 (negative control), 100, 180, 320, 560, and 1000 mg aqueous solution/L 0 (negative control), 35.7, 64.3, 114, 200, and 357 mg ai/L <LOQ (<2.50, control), 30.1 (low), 100.2 (medium), and 384.5 (high) mg Hoe 099730 sum of D and L enantiomers/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples from low, medium, and high test levels and the control were analyzed via HPLC with UV detection (202 nm). Fortification samples, and matrix and solvent blanks were analyzed concurrently.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	24.6-25.9°C Continuous 159 µE·m^{-2}·s^{-1} Wide spectrum fluorescent lamps of universal white-type L25	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- Cell density - Biomass - Growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell density was determined using counting chambers (Schreck, Hofheim, Germany) and a microscope (Zeiss, Oberkochen, Germany). The study author did not report how biomass and growth rate values were calculated.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None.	
Indicate whether there was an exponential growth in the control	Yes; cell density was 206 x 10^4 cells/mL at 72 hours.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At 72 hours, cell density in the negative control averaged 206 x 10^4 cells/mL, which yielded inhibitions of -11, -21, 13, -5, and -5% as compared to the control. An EC_{50} value was not calculated for this endpoint.

At 72 hours, biomass in the negative control averaged 3853 x 10^4 cells/mL*h, yielding inhibitions of -5, -10, 14, -4, and -6% as compared to the control. The 72-hour EC_{50} value was >1000 mg ai/L (concentrations unadjusted for % purity of test solution) or >357 mg ai/L (adjusted for purity).

At 72 hours, the growth rate in the negative control averaged 0.078 hours-1, yielding inhibitions of -1, -1, 2, -1, and-1%. The 72-hour EC_{50} value was >1000 mg ai/L (concentrations unadjusted for % purity of test solution) or >357 mg ai/L (adjusted for purity).

The overall NOAEC value, based on the level at which no significant growth inhibition and no cell deformation was observed, was 1000 mg test substance/mL, which corresponded to 357 mg ai/L.

No phytotoxic effects were observed.

Table 3: Effect of Hoe 099730 Technical on algal growth of *Scenedesmus subspicatus*.

Treatment **Nominal** **mg ai/L**	**Initial cell Density (x 10^4 cells/mL)**	**Cell density (x 10^4 cells/mL) at**			
		24 hours	**48 hours**	**72 hours**	
				cell count	**% inhibition**
Negative control	1.0	3.6	56.3	206	N/A
35.7	1.0	4.1	51.9	229	-11
64.3	1.0	5.0	49.1	250	-21
114	1.0	2.9	48.1	180	13
200	1.0	3.5	57.4	217	-5
357	1.0	4.2	60	217	-5
Reference chemical (if used)	N/A				

Table 4: Effect of Hoe 099730 Technical on algal growth (*Scenedesmus subspicatus*).

Treatment Nominal mg ai/L	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (hours^{-1})		Mean Biomass ($x\ 10^4$ cells/mL*h)	
		0-72 Hours	Percent Inhibition	0-72 hours	Percent Inhibition
Negative control	1.0	0.0780	N/A	3853	N/A
35.7	1.0	0.0786	-1	4034	-5
64.3	1.0	0.0787	-1	4239	-10
114	1.0	0.0766	2	3321	14
200	1.0	0.0789	-1	4004	-4
357	1.0	0.0784	-1	4090	-6

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell density	Growth rate	Biomass
NOAEC or EC_{05} (mg ai/L)	ND	357	357
EC_{50} (mg ai/L) (95% C.I.)	ND	>357	>357
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A		

* Do not use this table, if the study was deemed unacceptable.

B. REPORTED STATISTICS:

The EC_{50} values for biomass and growth rate could not be determined due to a lack of an inhibitory effect of ≥50%. The cell density data were not analyzed. The NOAEC was determined using ANOVA and General Linear Models in SAS via Duncan's Multiple Range Test Procedures. Nominal concentrations were used for analysis.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer tested cell density and biomass replicate data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Levene's test in Toxstat 3.5. All endpoint data met the assumptions of ANOVA, and were thus analyzed using the Bonferroni t-test and Williams' tests to determine the NOAEC. The toxicity values for growth rate data were visually determined due to inhibitions that were <5%. The ECx values (with 95% C.I.) and probit slope could not be determined due to a lack of an inhibitory effect of ≥50%.

All toxicity values were determined using a combination of the available measured concentrations and the 72-hour nominal concentrations adjusted for the percent purity (for the concentrations that were not analytically determined). Cell density values were entered into Toxstat 3.5 as an abbreviated value, representing the value x 10^4.

Cell density
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

Analytical verification was not performed for all test levels.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement. However, the reviewer analyzed cell density, while the study author did not attempt statistical analysis. Therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The experiment was started February 8, 1994, and was terminated February 25, 1994.

F. CONCLUSIONS:

This toxicity study is classified as scientifically sound and is classified as **supplemental**. Although consistent with OECD test guideline 201, it does not satisfy OCSPP guideline 850.5400 because the algae in controls had not reached the logarithmic growth phase by test termination and the light intensity was much higher than what is recommended in the OCSPP guideline. These deviations may affect the nature of the growth curve and the concentration-response, thereby limiting the utility of the information in EPA risk assessment. However, EFED is not requesting additional algal toxicity tests with glufosinate degradates at this time. The most sensitive endpoint could not be determined due to a lack of toxicity in this study, resulting in overall NOAEC and EC_{50} values of 384 and >384 mg ai/L, respectively.

Cell density
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >384 mg ai/L 95% C.I.: N/A
EC_{50}: >384 mg ai/L 95% C.I.: N/A
NOAEC: 384 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

Organization for Economic Cooperation and Development, 1984. OECD Guidelines for Testing of Chemicals. Guideline No. 201: Alga, Growth Inhibition Test. 07 June 1984.

Kuhl, A. and Lorenzen, H., 1964. Handling and Culturing of Chlorella, in Methods of Cell Physiology, Vol. I, New York – London, pp. 159-187.

U.S. Environmental Protection Agency (EPA), 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants.

U.S. Environmental Protection Agency (EPA), 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792). Fed. Reg.,Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc., 1989, Release 6.07 TS 305. SAS Institute Inc., Cary, North Carolina 27511.

Microsoft EXCEL, 1993. Frontline Systems, Inc., P.O. Box 4288, Incline Village, Nevada 89450-4288.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
File:             4813c             Transform:              NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------------

     D = 11714.7267
     W =   0.9255

     Critical W  = 0.8730 (alpha = 0.01 , N = 21)
              W  = 0.9080 (alpha = 0.05 , N = 21)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.



Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
File:             4813c             Transform:              NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5           1275.9857         255.1971       0.6756

   Within (Error)     15           5665.9467         377.7298
--------------------------------------------------------------------------------
   Total              20           6941.9324
--------------------------------------------------------------------------------
                                                        (p-value = 0.6484)

   Critical F  = 4.5556  (alpha = 0.01, df = 5,15)
               = 2.9013  (alpha = 0.05, df = 5,15)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)



Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
File:             4813c             Transform:              NO TRANSFORMATION

                              ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5           8498.1914        1699.6383       2.1763
```

```
   Within (Error)     15          11714.7267         780.9818
  ------------------------------------------------------------------------------
   Total              20          20212.9181
  ------------------------------------------------------------------------------
                                                              (p-value = 0.1117)

   Critical F  = 4.5556  (alpha = 0.01, df = 5,15)
               = 2.9013  (alpha = 0.05, df = 5,15)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.05)


  Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
  File:             4813c             Transform:             NO TRANSFORMATION

       Bonferroni t-Test   -   TABLE 1 OF 2            Ho: Control<Treatment
  ------------------------------------------------------------------------------
                                TRANSFORMED    MEAN CALCULATED IN          SIG
  GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    t STAT
0.05
  ----- --------------------    -----------    ------------------  ------  ---
    1            Neg control    206.2333           206.2333
    2                   30.1    229.2000           229.2000       -1.1622
    3                   64.3    250.0000           250.0000       -2.2148
    4                    100    179.7333           179.7333        1.3410
    5                    200    216.8000           216.8000       -0.5347
    6                    384    217.3333           217.3333       -0.5617
  ------------------------------------------------------------------------------
  Bonferroni t critical value = 2.6025   (1 Tailed, alpha = 0.05, df = 5,15)


  Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
  File:             4813c             Transform:             NO TRANSFORMATION

       Bonferroni t-Test   -   TABLE 2 OF 2            Ho: Control<Treatment
  ------------------------------------------------------------------------------
                              NUM OF     MIN SIG DIFF     % OF     DIFFERENCE
  GROUP    IDENTIFICATION      REPS    (IN ORIG. UNITS)  CONTROL  FROM CONTROL
  ----- --------------------  -------  ----------------  -------  ------------
    1            Neg control     6
    2                   30.1     3           51.4272       24.9     -22.9667
    3                   64.3     3           51.4272       24.9     -43.7667
    4                    100     3           51.4272       24.9      26.5000
    5                    200     3           51.4272       24.9     -10.5667
    6                    384     3           51.4272       24.9     -11.1000
  ------------------------------------------------------------------------------


  Title:  Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
  File:             4813c             Transform:             NO TRANSFORMATION
```

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	206.2333	206.2333	222.9167
2	30.1	3	229.2000	229.2000	222.9167
3	64.3	3	250.0000	250.0000	222.9167
4	100	3	179.7333	179.7333	204.6222
5	200	3	216.8000	216.8000	204.6222
6	384	3	217.3333	217.3333	204.6222

Title: Hoe 099730 & S. subspicatus 72-hr cell density; mg ai/L
File: 4813c Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	206.2333				
30.1	222.9167	-0.8443		1.7500	k= 1, v=15
64.3	222.9167	-0.8443		1.8400	k= 2, v=15
100	204.6222	0.0815		1.8700	k= 3, v=15
200	204.6222	0.0815		1.8800	k= 4, v=15
384	204.6222	0.0815		1.8900	k= 5, v=15

s = 27.9461

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 2347990.1667
W = 0.9855

Critical W = 0.8730 (alpha = 0.01 , N = 21)
W = 0.9080 (alpha = 0.05 , N = 21)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	331642.9762	66328.5952	1.3484
Within (Error)	15	737864.8333	49190.9889	
Total	20	1069507.8095		

(p-value = 0.2978)

Critical F = 4.5556 (alpha = 0.01, df = 5,15)
= 2.9013 (alpha = 0.05, df = 5,15)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	1555042.9764	311008.5953	1.9869
Within (Error)	15	2347990.1665	156532.6778	
Total	20	3903033.1429		

(p-value = 0.1392)

Critical F = 4.5556 (alpha = 0.01, df = 5,15)
= 2.9013 (alpha = 0.05, df = 5,15)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	Neg control	3853.1667	3853.1667		
2	30.1	4034.3333	4034.3333	-0.6476	
3	64.3	4239.0000	4239.0000	-1.3792	
4	100	3321.0000	3321.0000	1.9022	
5	200	4004.0000	4004.0000	-0.5392	
6	384	4090.3333	4090.3333	-0.8477	

Bonferroni t critical value = 2.6025 (1 Tailed, alpha = 0.05, df = 5,15)

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	30.1	3	728.0731	18.9	-181.1667
3	64.3	3	728.0731	18.9	-385.8333
4	100	3	728.0731	18.9	532.1667
5	200	3	728.0731	18.9	-150.8333
6	384	3	728.0731	18.9	-237.1667

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L
File: 4813b Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	3853.1667	3853.1667	3994.9167
2	30.1	3	4034.3333	4034.3333	3994.9167
3	64.3	3	4239.0000	4239.0000	3994.9167
4	100	3	3321.0000	3321.0000	3805.1111
5	200	3	4004.0000	4004.0000	3805.1111
6	384	3	4090.3333	4090.3333	3805.1111

Title: Hoe 099730 & S. subspicatus 72-hr biomass; mg ai/L

File: 4813b Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	3853.1667				
30.1	3994.9167	-0.5067		1.7500	k= 1, v=15
64.3	3994.9167	-0.5067		1.8400	k= 2, v=15
100	3805.1111	0.1718		1.8700	k= 3, v=15
200	3805.1111	0.1718		1.8800	k= 4, v=15
384	3805.1111	0.1718		1.8900	k= 5, v=15

s = 395.6421

WARNING: Procedure has used isotonized means which differ from original (transformed) means.